

10054712.41304

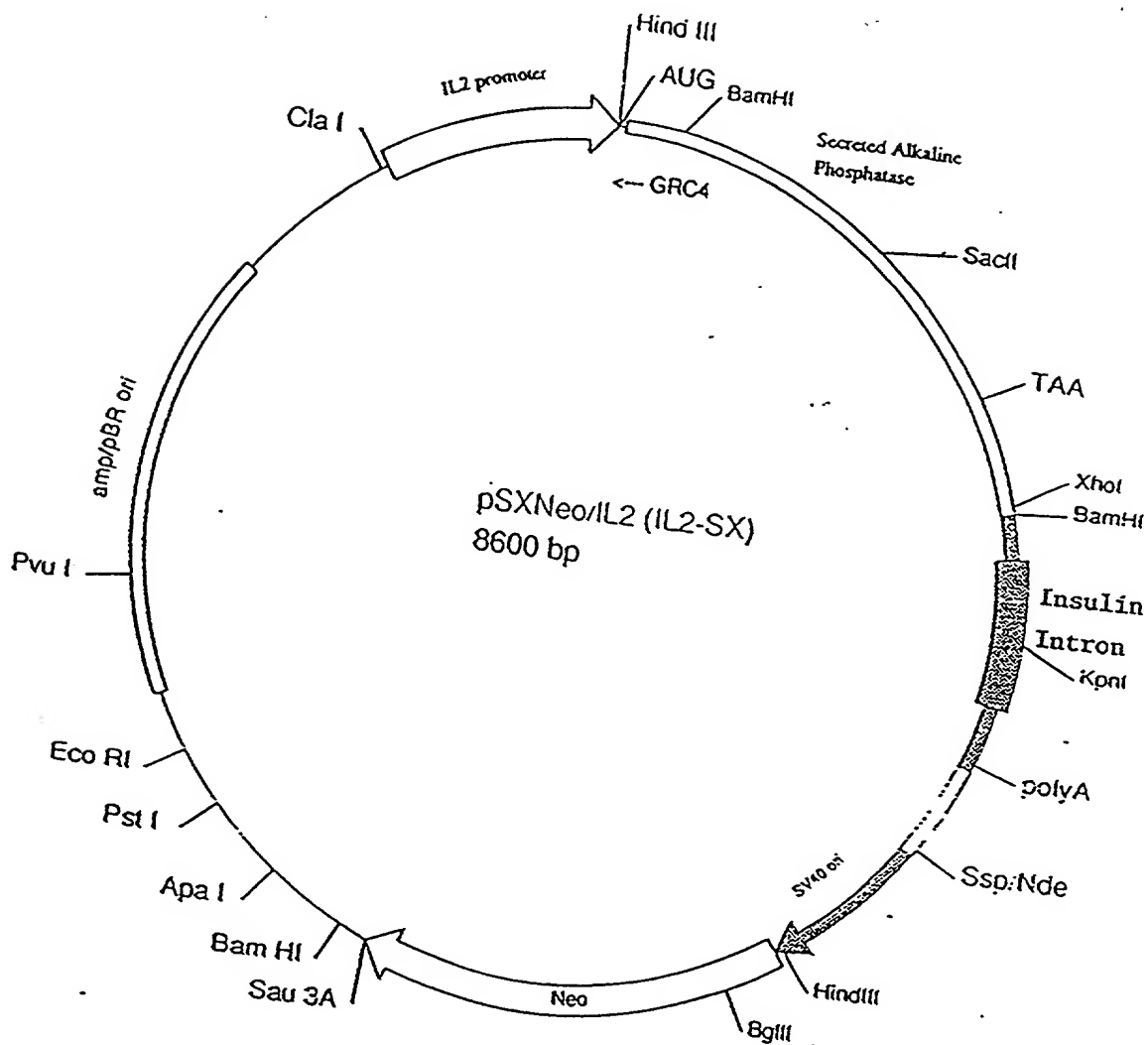
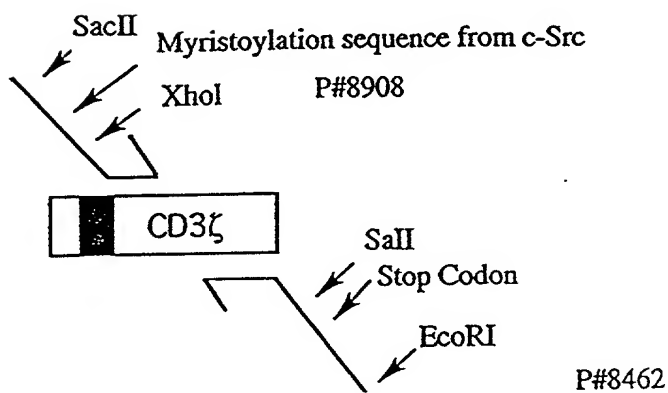


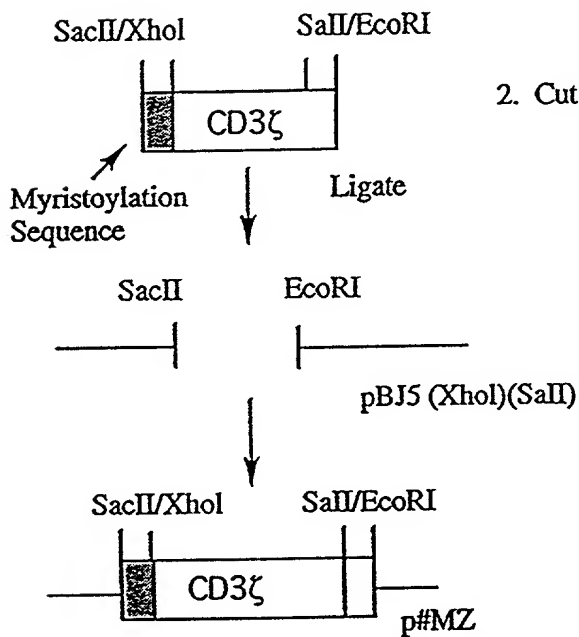
Figure 1/21

Construction of intracellular signalling chimera:

1. PCR myristoylated CD3 ζ

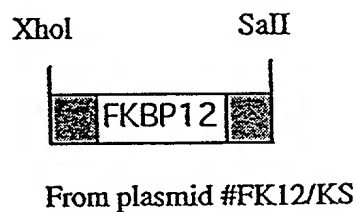


2. Cut and clone PCR fragment

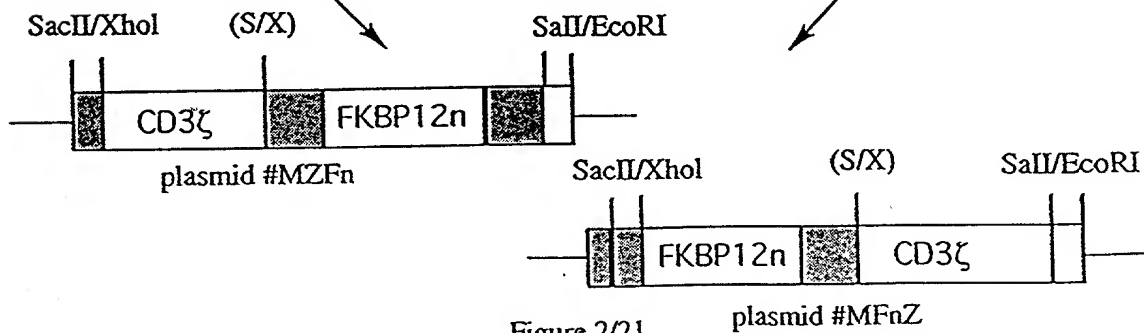


*The MZE series contains a 9aa HA epitope at the 3' end.

3. SEQUENCE insert



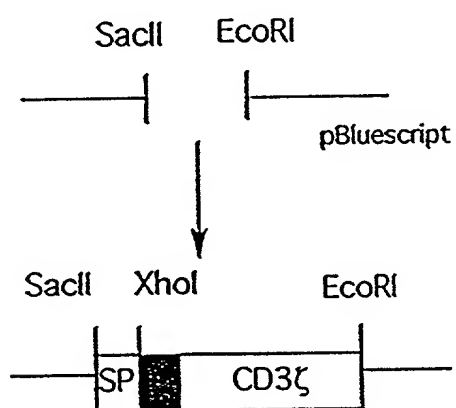
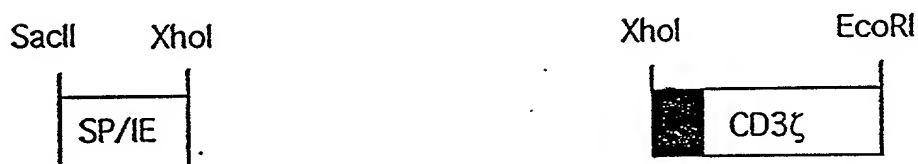
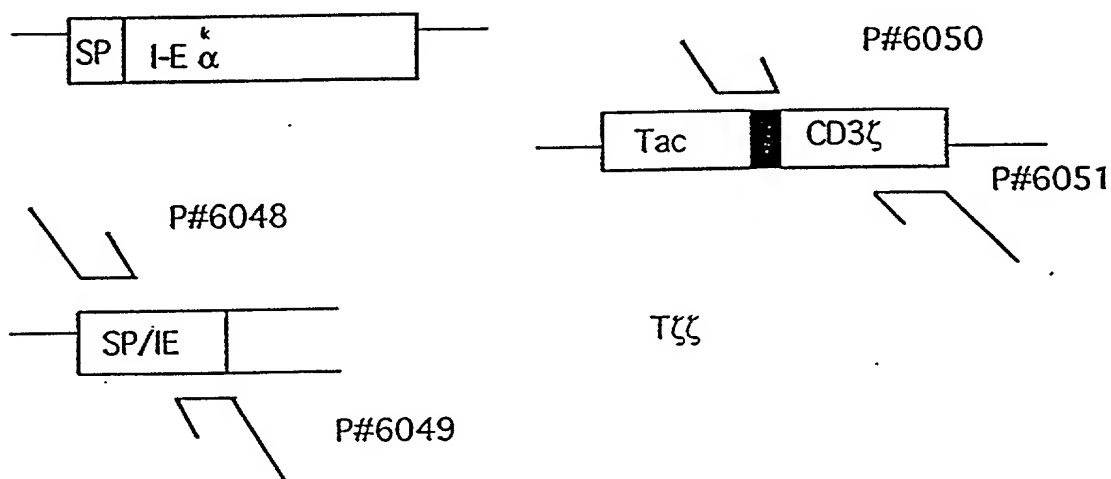
4. Cut at XhoI or SacII and add FKBP domains



Construction of extracellular signaling chimera:

1. PCR murine signal peptide

2. PCR CD3 trans-membrane and cytoplasmic domains



plasmid #SPZ/KS
SEQUENCE insert*

Cut XhoI

10544-4307

10544-4307

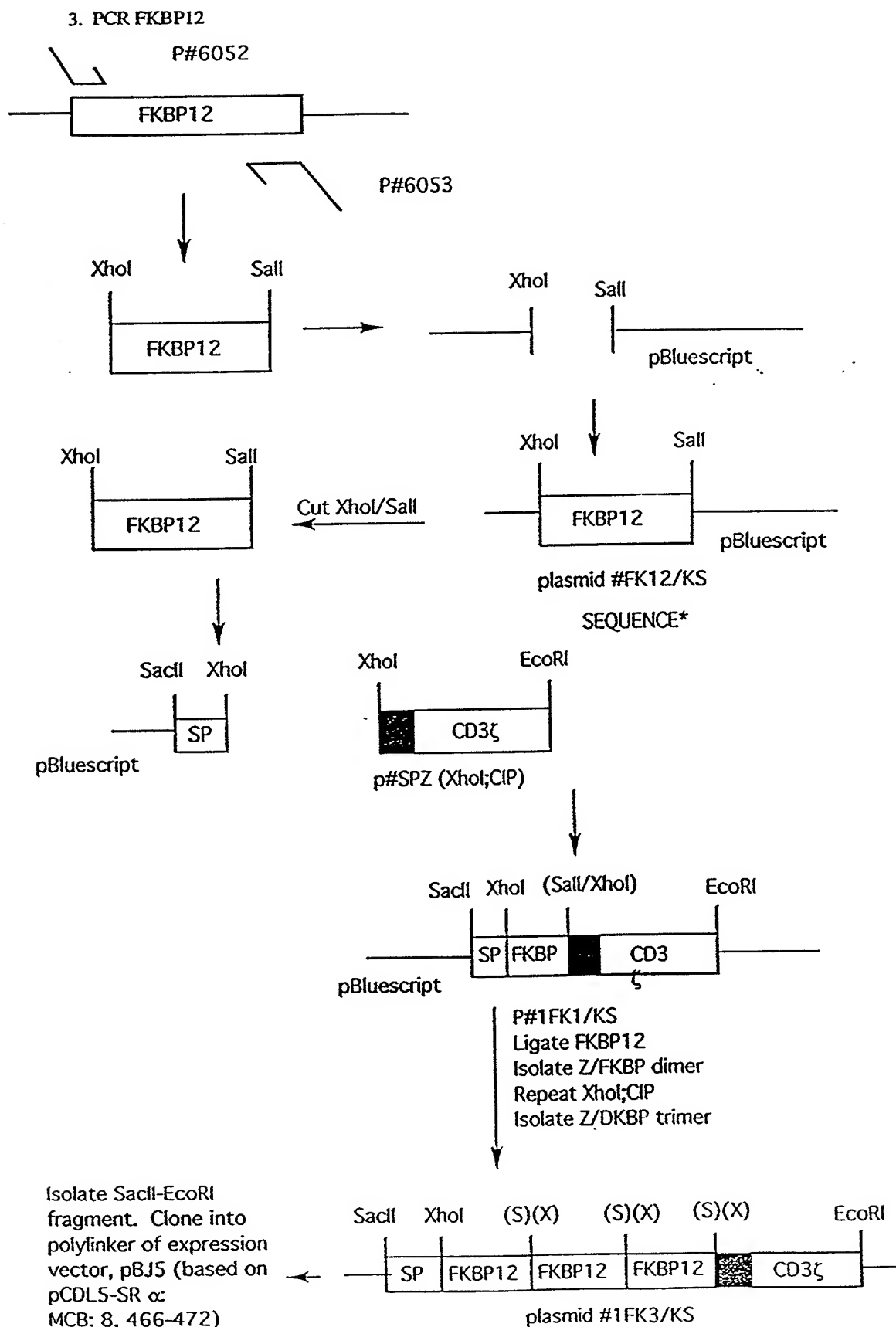


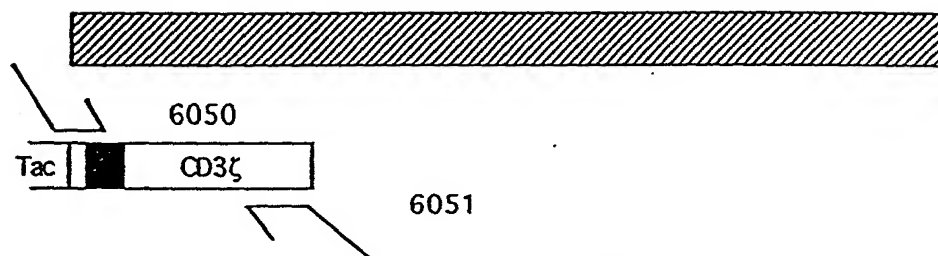
Figure 3B/21

6048: 5'-CGACACCGCGGCCACCATGGCCACAATTGGAGC-3'

SacII homology

Kozak M A T I G

6049: 5'-CGACACTCGAGAGCCCATGACTTCTGG
L A W S - 7



Asp-Gyl#1

6050: 5'CGACACTCGAGCTCTGCTACTTGCTAGGTGGAATCCTCTTC-3'

E L C Y L L G G I L F

*A to G

6051: 5' GCGAATTCTTAGCGAGGGGCCAGC-3' *G to C

EcoRI homology

St R P A L

3'Sal #B

8462: 5' GCGAATTCTTAGTCGACGCGAGGGGCCAGGGTC-3'

 St R PAL

 EcoRI SalI homology

Cys-Gly #2

7129: 5' GGGCTCGAGCTCGGCTACTTGCTAG-3'

homology

XhoI *

L G Y L L

*T to G

CYCC

6568: 5'-CGACACTCGAGGTGACGGACAAGGTC-3'
XhoI homology

6569: 5'-CGACAGTCGACCCAATCAGGGACCTC-3'
Sall homology

EPITOPE

7850: 5'-TCGAGTATCCGTACGACGTACCAGACTACGCAG-3'
XhoI BsiWI
Y P Y D V P D Y A

7851: 5'-TCGACTGCGTAGTCTGGTACGTCGTACGGATAC-3'
Sall

EPITOPE: 5SEP, 3XEP

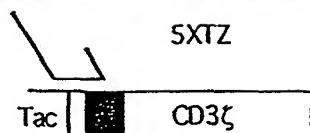
8922: 5'-TCGACTATCCGTACGACGTACCAGACTACGCAC-3'
Sall

8923: 5'-TCGAGTGCGTAGTCTGGTACGTCGTACGGATAG-3'
XhoI

Myristoylation from c-src SSMXZ

8908: 5'-CGACACCGCGGCCACCATGGGGAGTAGCAAGAGCAAGCCT
SacII
KOZAK M G S S K S K P

AAGGACCCCAGCCAGCGCCTCGAGAGGAGTGCAGAGACTG-3'
XhoI ζ-homology
K D P S Q R L E R S A E T



8912: 5'-CGACACTCGAGGAGCTCTGTGACGATG-3'
XhoI homology
E L C D D

Figure 4B/21

Asp-Lys #4
 8061: 5'-CGACACTCGAGCTCTGCTACTTGCTAAAGGGAATCCTCTTC-3'
 E L C Y L L K G I L F

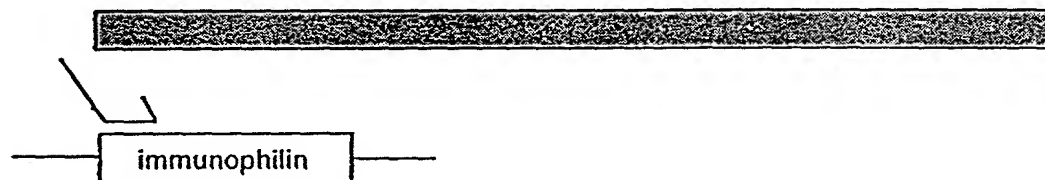
XhoI [homology] **

#4 Extension
 8907: 5'-CGACACTCGAGCTGCTGGATCCGAAGCTCTGCTACTTGCTAAAG-3'
 E L L D P K L C Y L L K

XhoI [homology] *GATtoAAG

TAC-Tm #3
 7220: 5'-CGACACTCGAGACAACAGAGTACCAGGTAGC-3'
 E T T E Y Q V

XhoI [homology]



FKBP12

6052: 5'-CGACACTCGAGGGCGTGCAGGTGGAGAC-3'
 E G V Q V E

XhoI [homology]

6053: 5'-CGACAGTCGACTTCCAGTTT TAGAAGC-3'
 V E L K L L

Sall [homology]

FKBP13

8460: 5'-TCGACACTCGAGACGGGGGCCGAGGGC-3'
 E T G A E G

XhoI [homology]

8461: 5'-CCGACAGTCGACCTCTATTTT GAGCAGC-3'
 V E I

Sall [homology]

Figure 4C/21

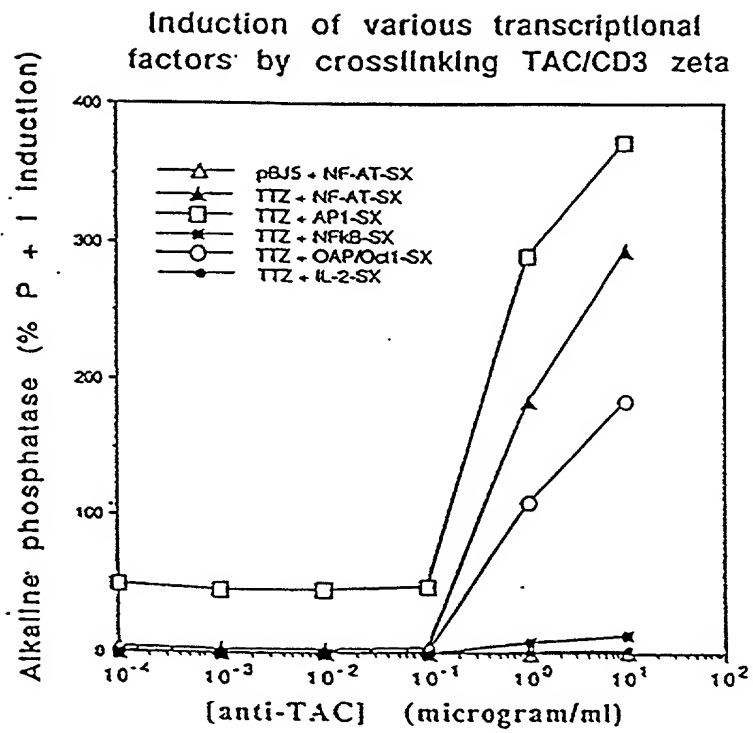


Figure 5/21

Inhibitory activity of dimeric FK506 and CSA

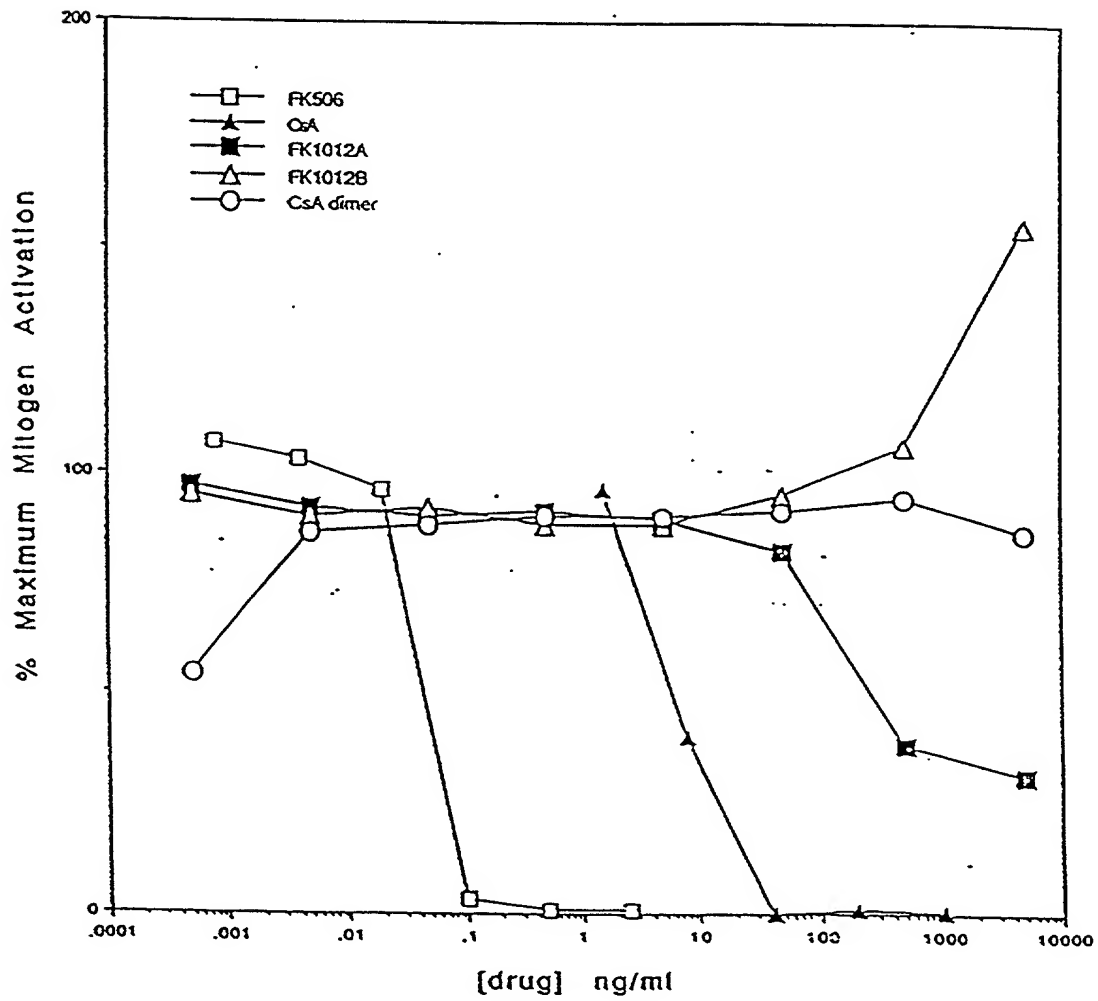
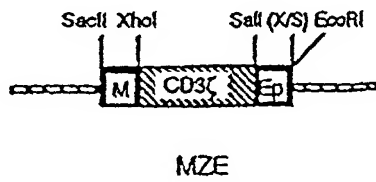
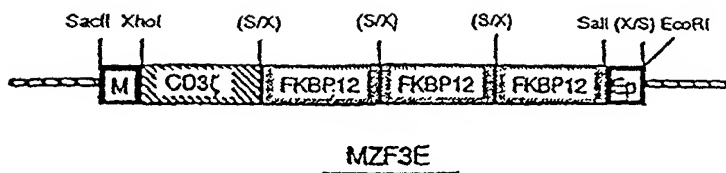
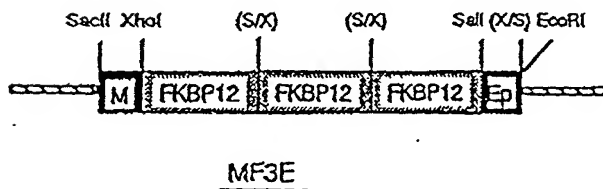


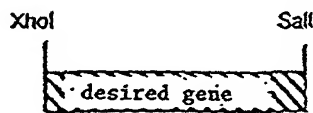
Figure 6A/21



Cut XhoI/Sall; CIP; + FKBP12 X 3



+



1. Cytoplasmic moiety of surface receptor
2. Tyrosine Kinase
3. Transcription Factor
4. Others

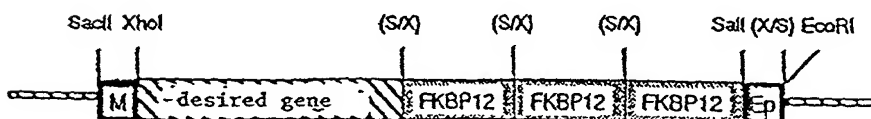


Figure 6B/21

Activity of FK1012A on the chimeric FKBPX3/CD3 zeta receptor

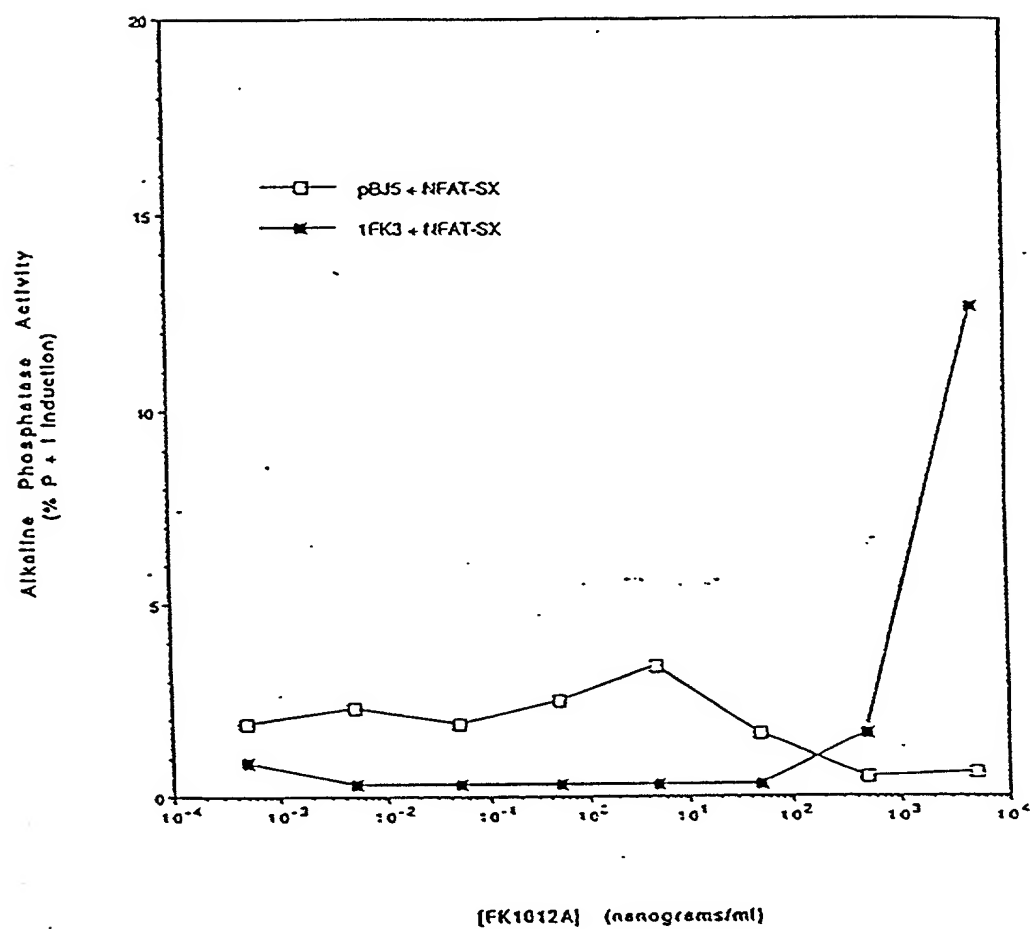


Figure 7/21

100547-21-45001

Activation of an NFAT reporter via
signalling through a myristoylated
CD3 zeta/FKBP12 chimera

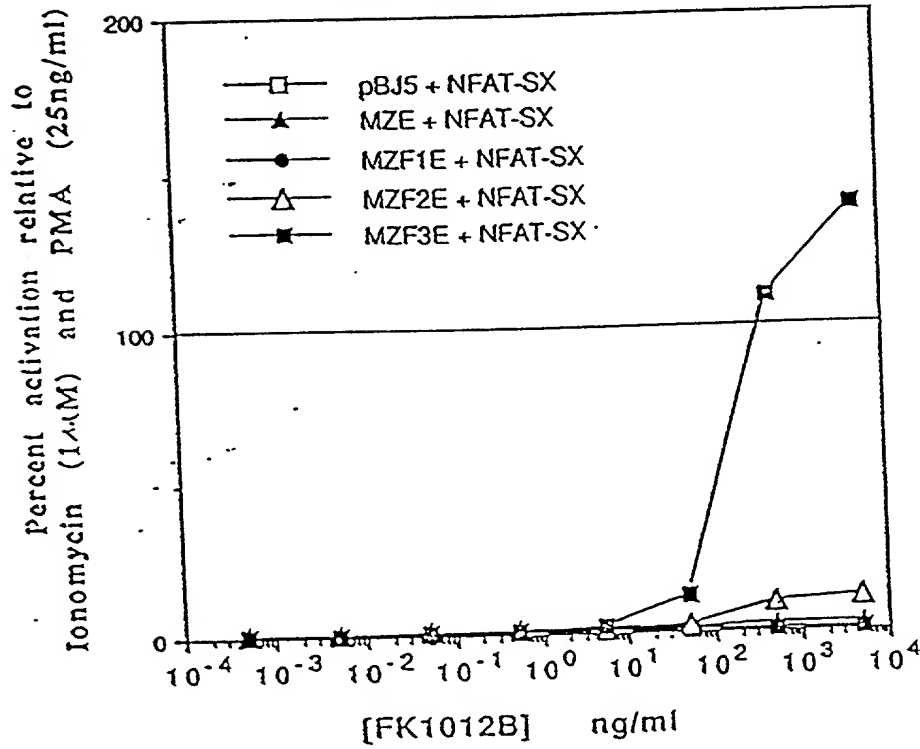


Figure 8/21

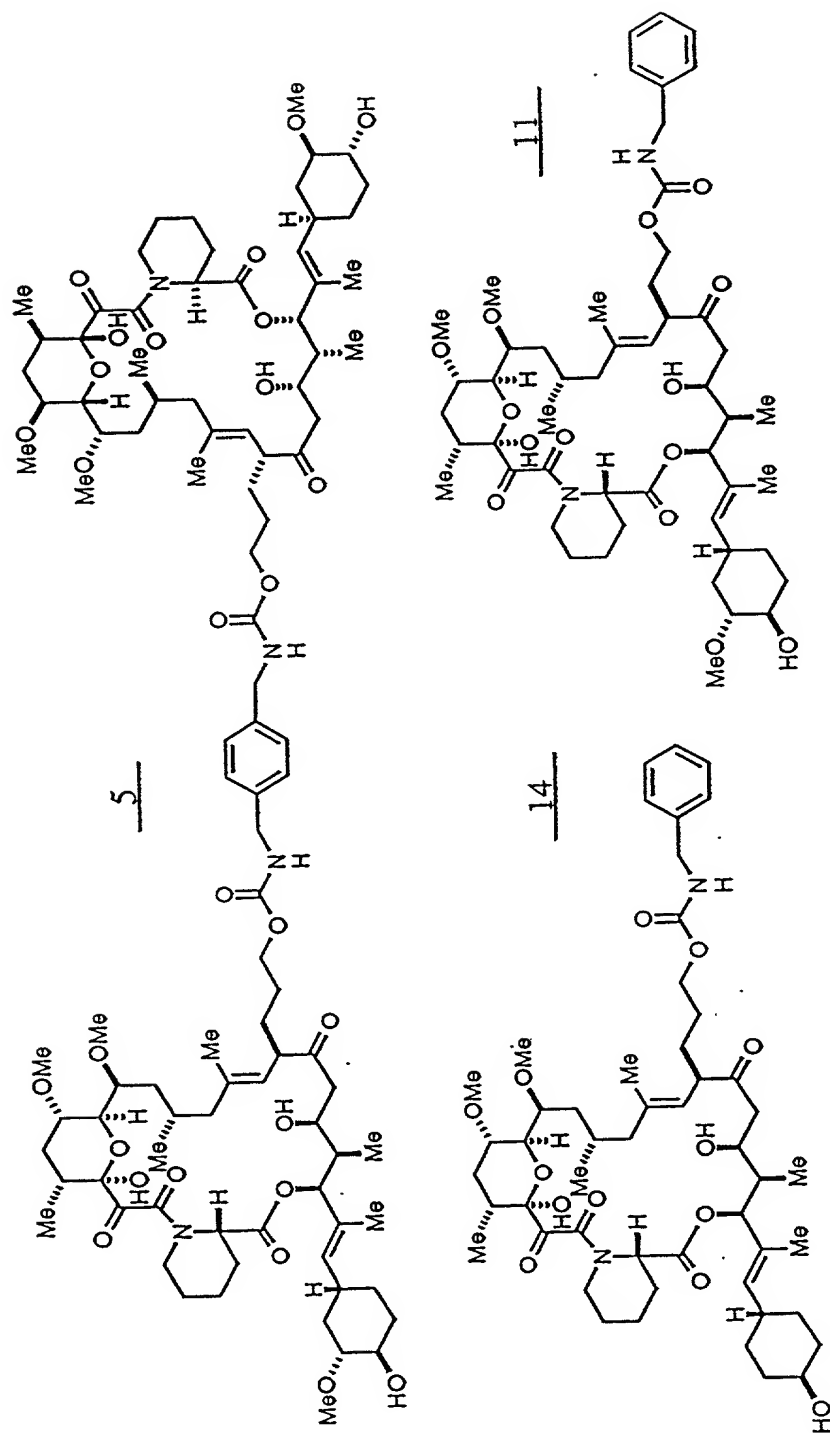


Figure 9A (#1)/21

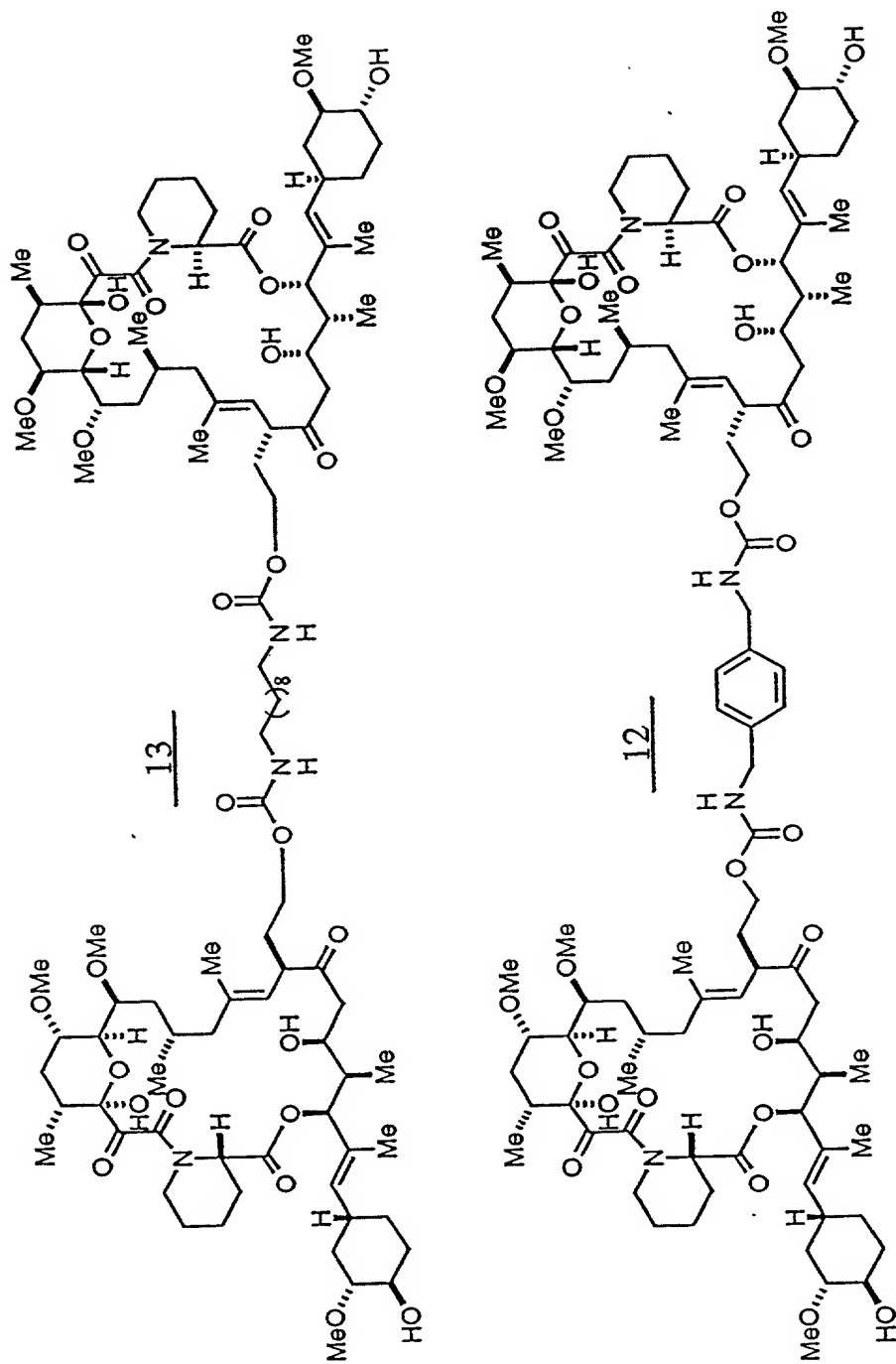


Figure 9A(#2)/21

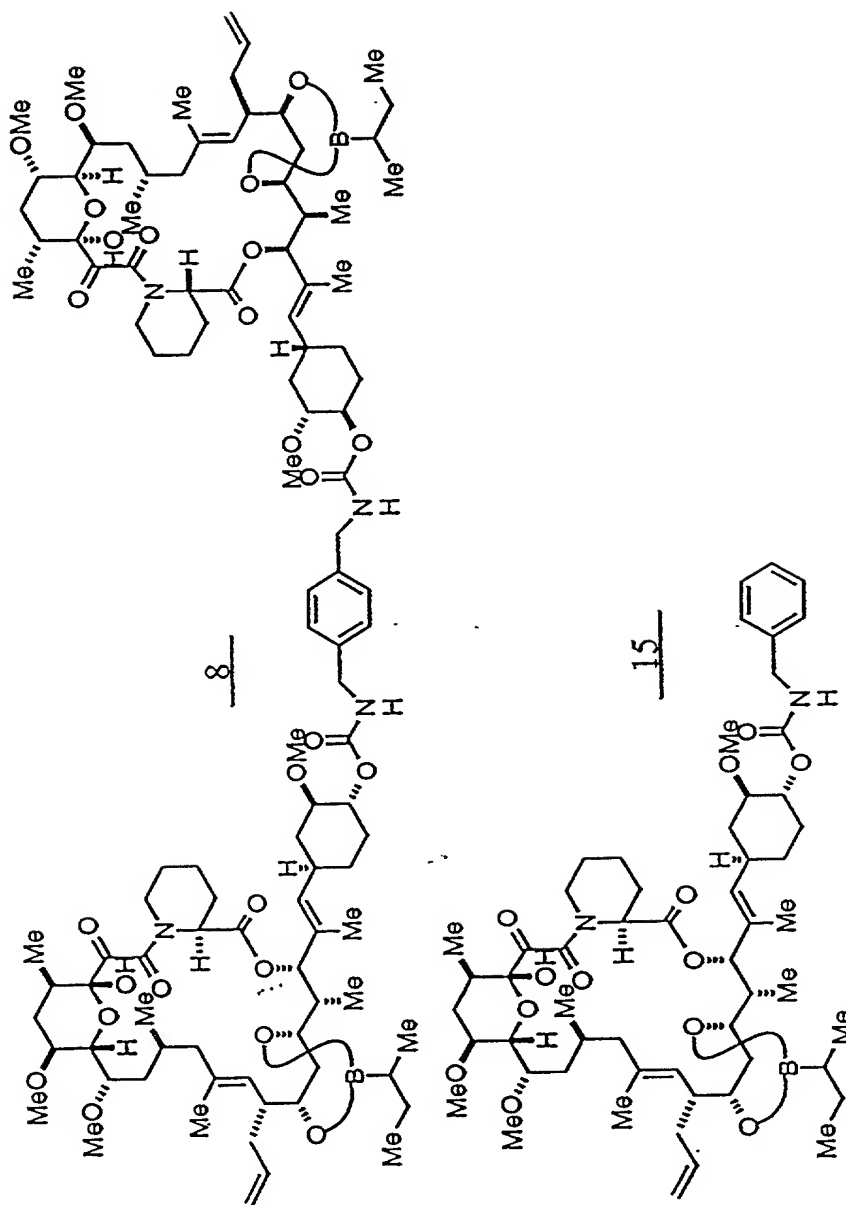
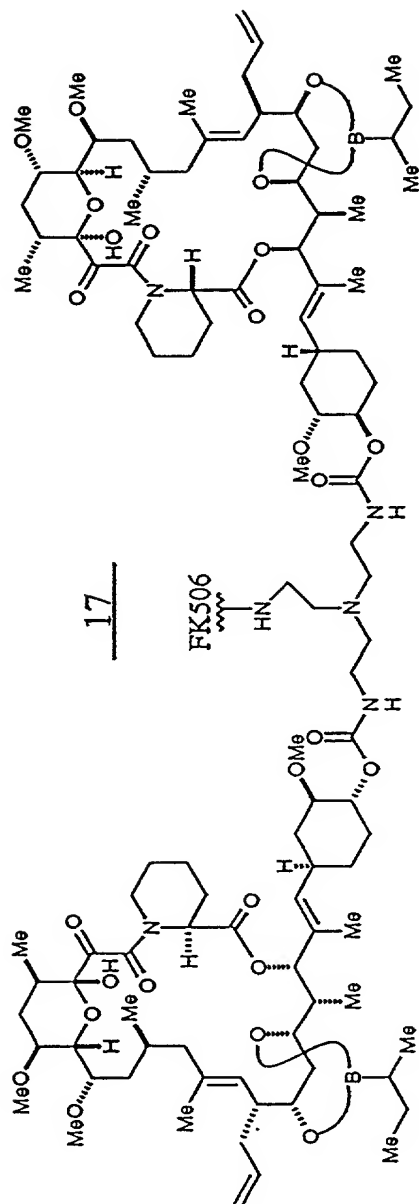
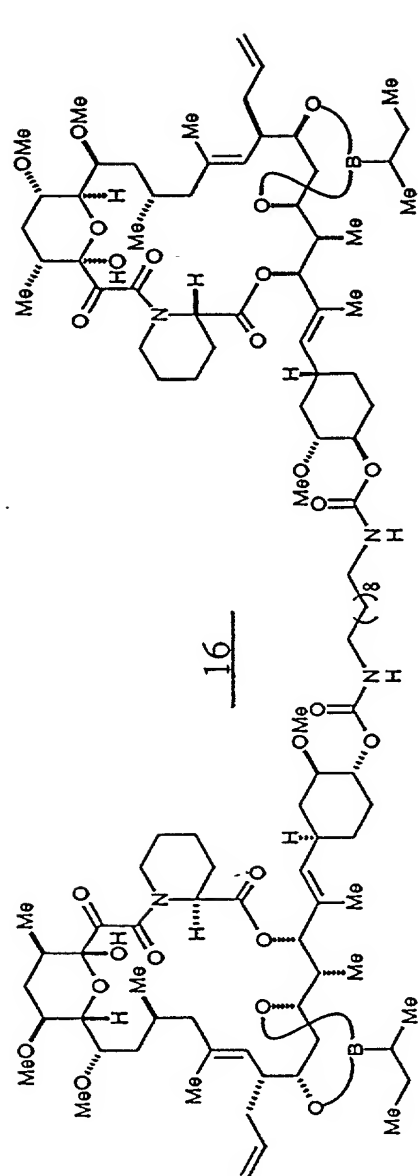


Figure 9B (#1)/21



FK506

Figure 9B (#2)/21

Scheme 1

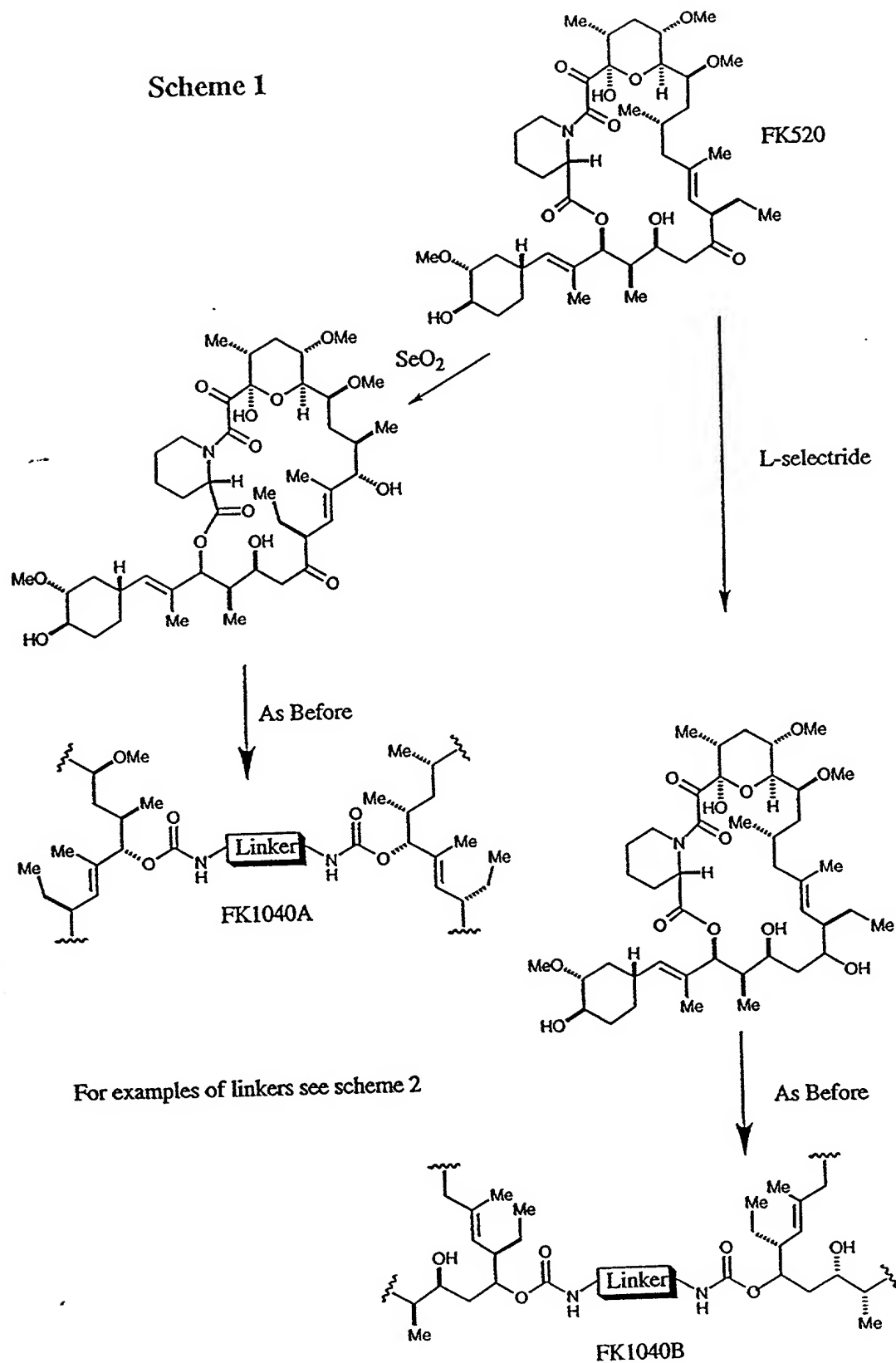


Figure 10/21

Scheme 2: Synthesis of Dimers

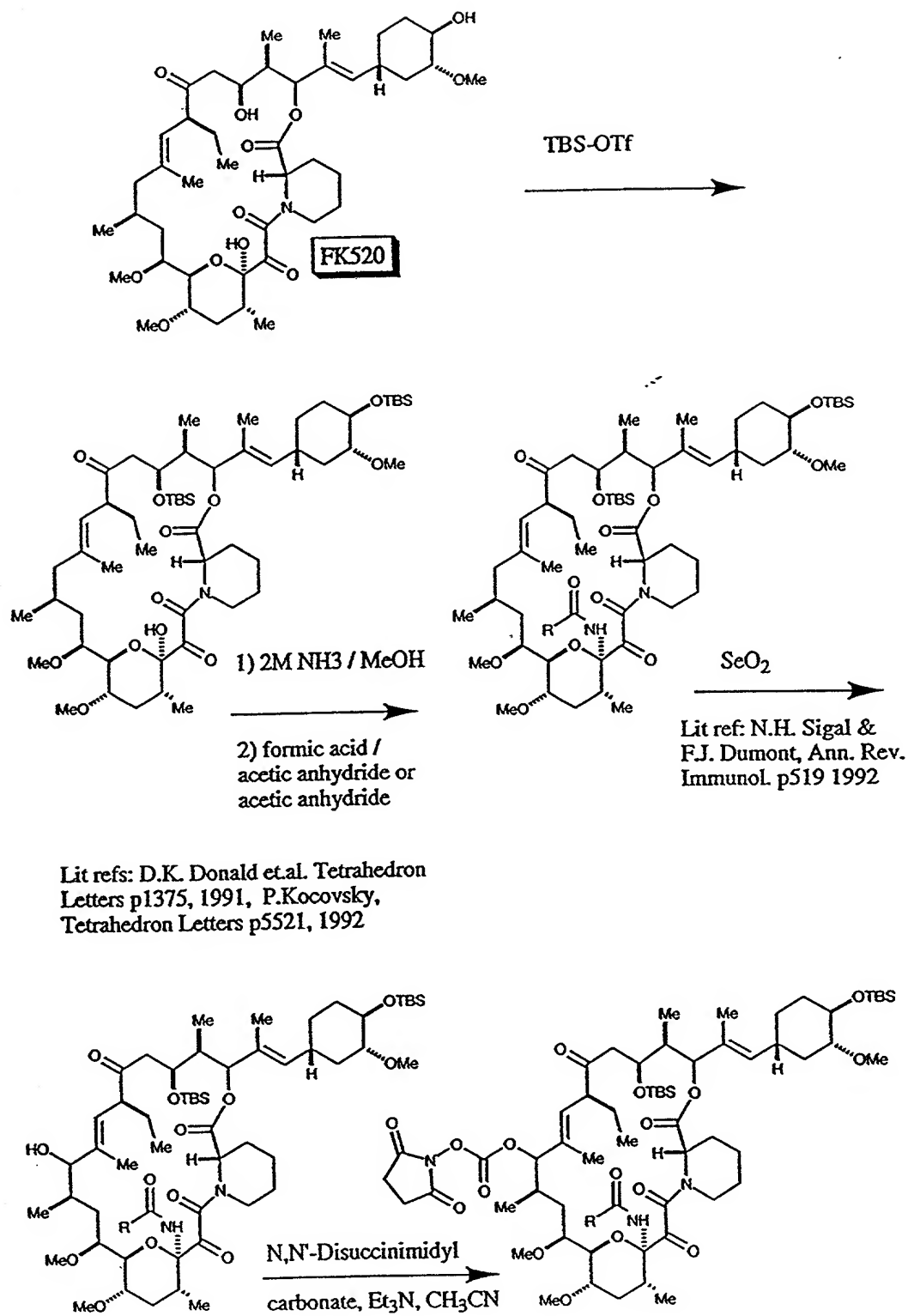


Figure 11A/21

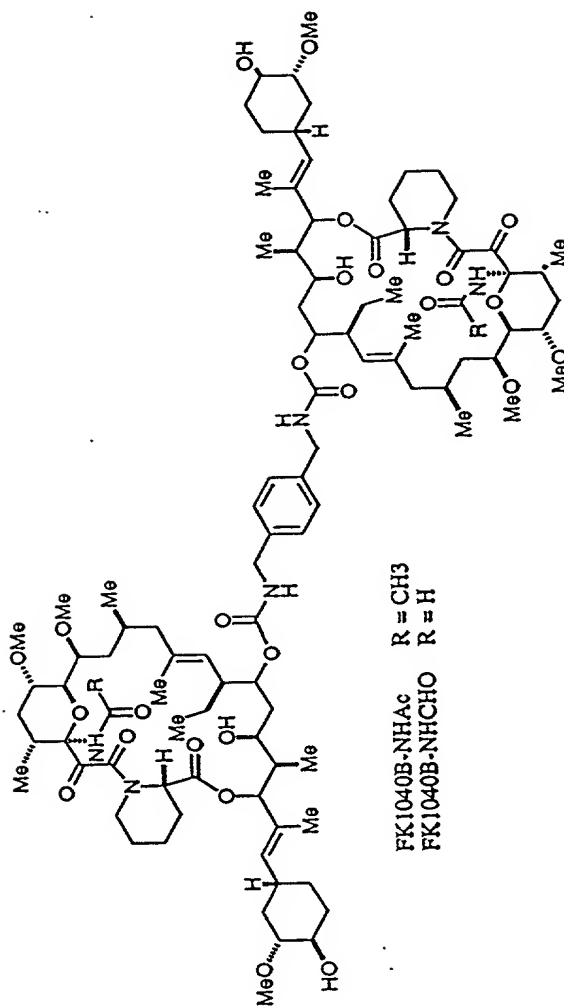
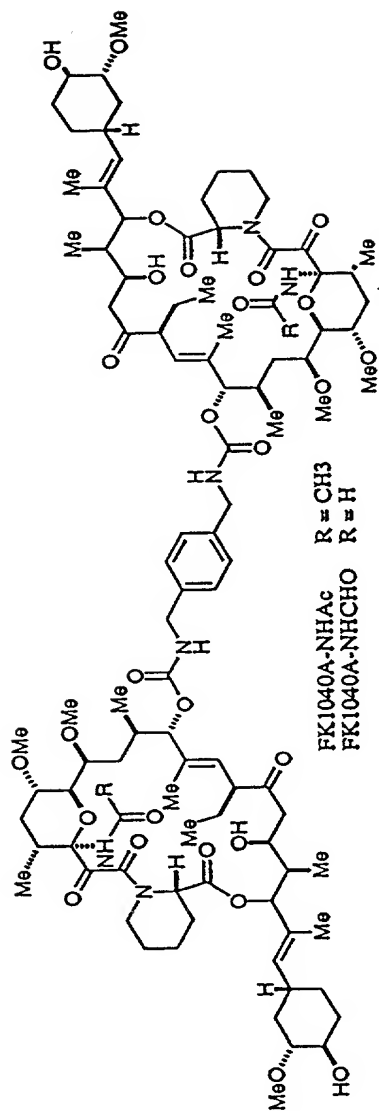


Figure 11B (#1)/21

An additional modified FK520 (FK1040) that interferes with FKBP12 yet should bind the FKBP12 mutant:
F36A or F99A or Y26A, or combinations thereof is

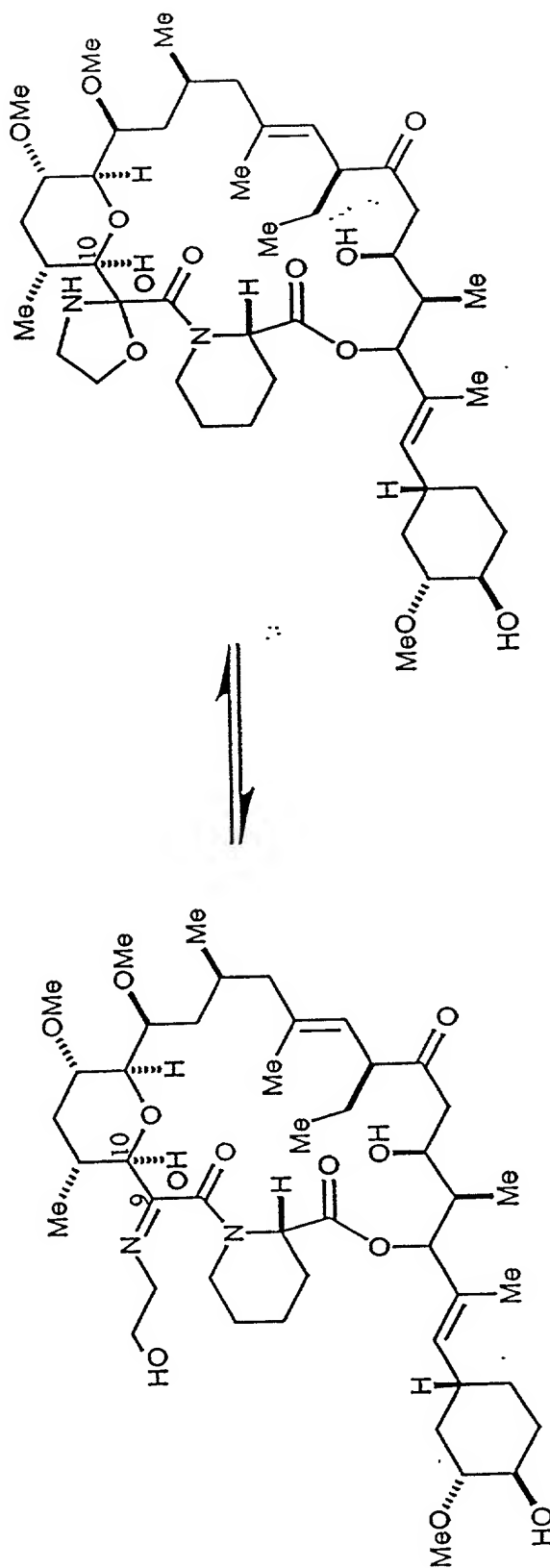


Figure 11B (#2)/21

Scheme 3 Heterodimerization

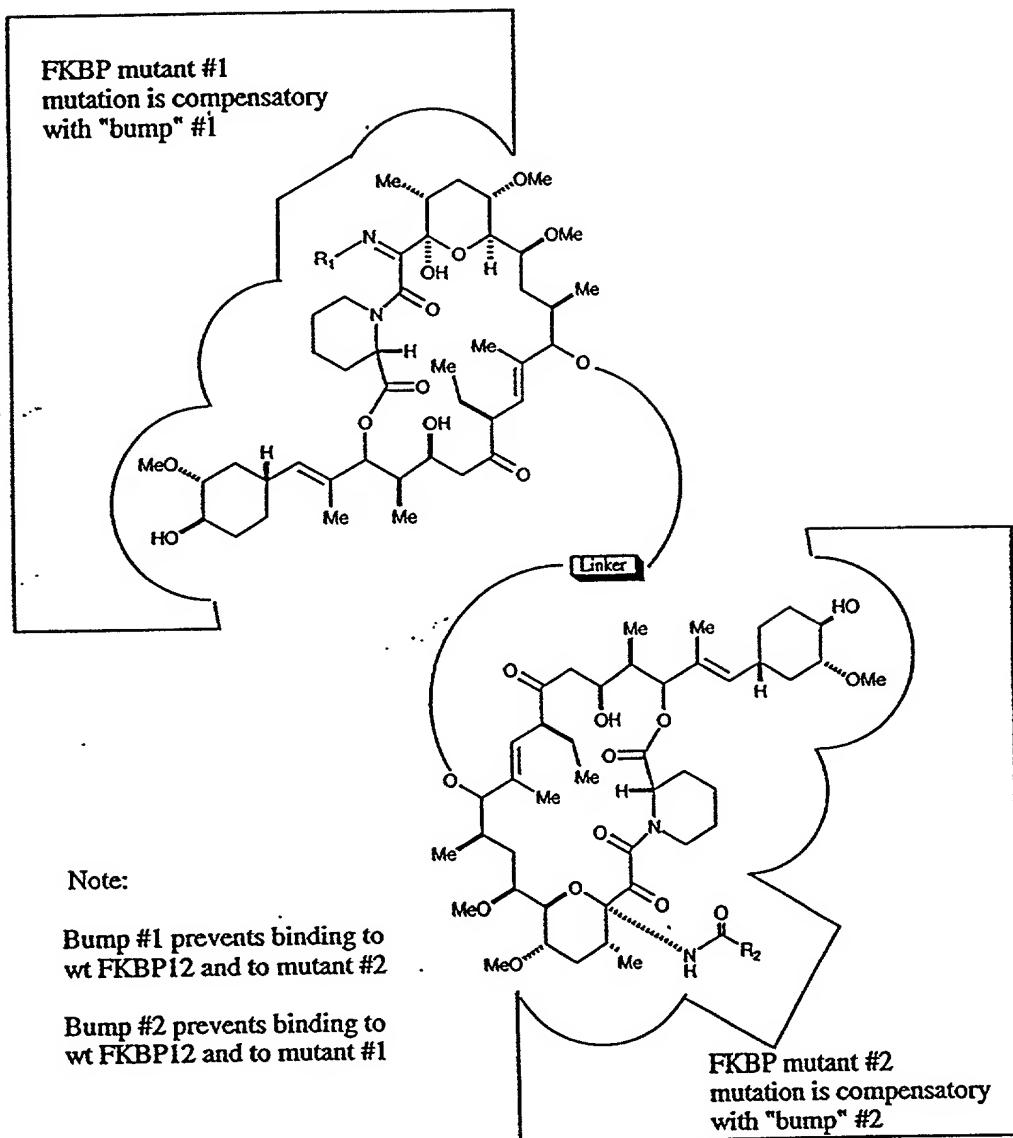
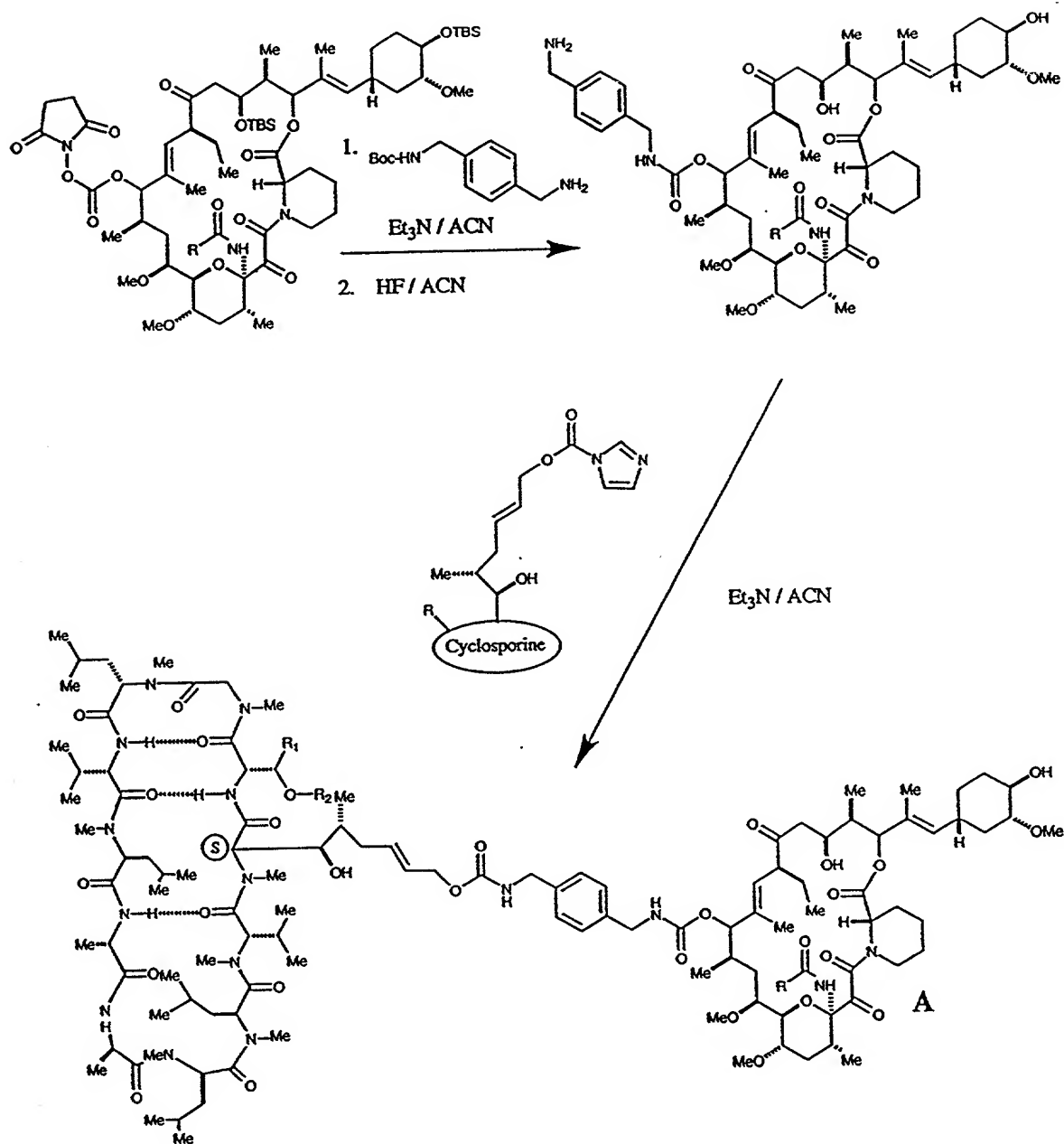


Figure 12/21

Scheme 3: Synthesis of heterodimers



In this example, a heterodimer of a cyclosporine analog and FK520A-NHCO-R were heterodimerized. However, the scheme can easily incorporate other FK506/520 derivatives to form hetero or homodimers

Figure 13/21

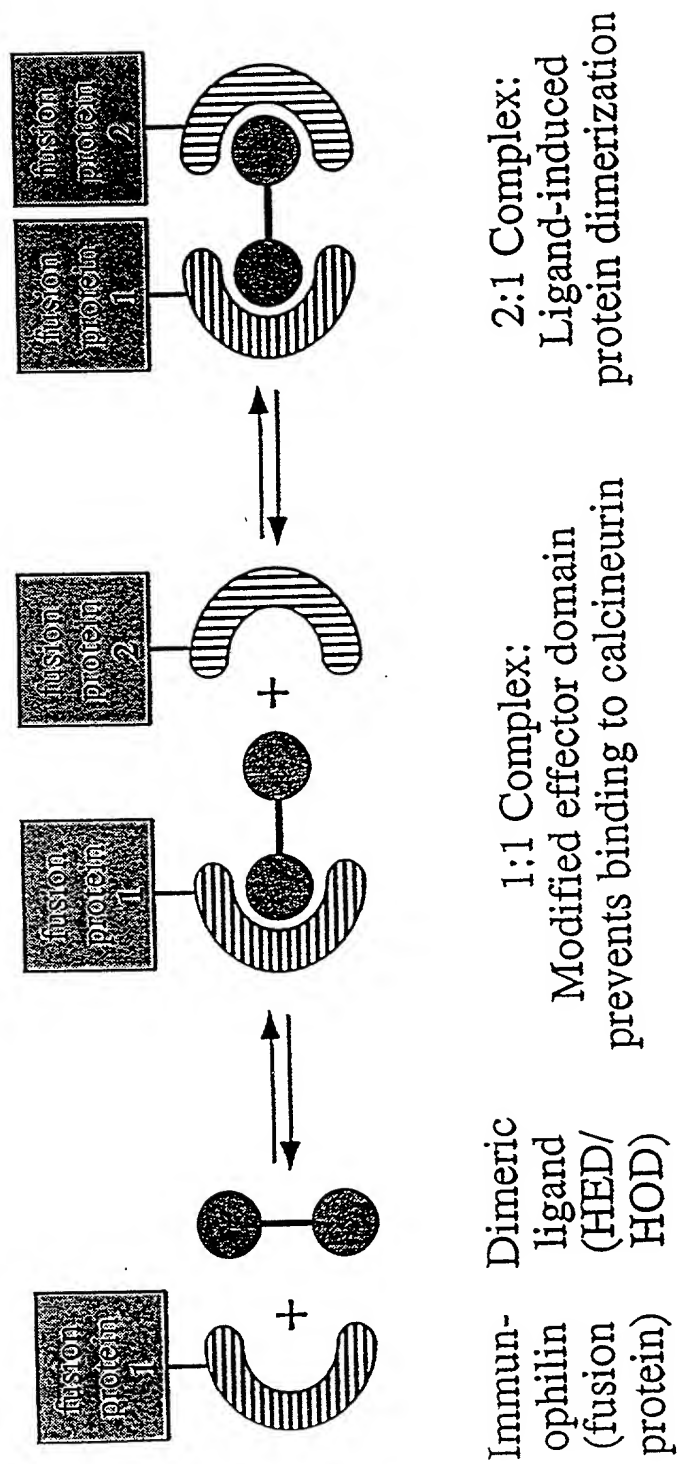


Figure 14/21

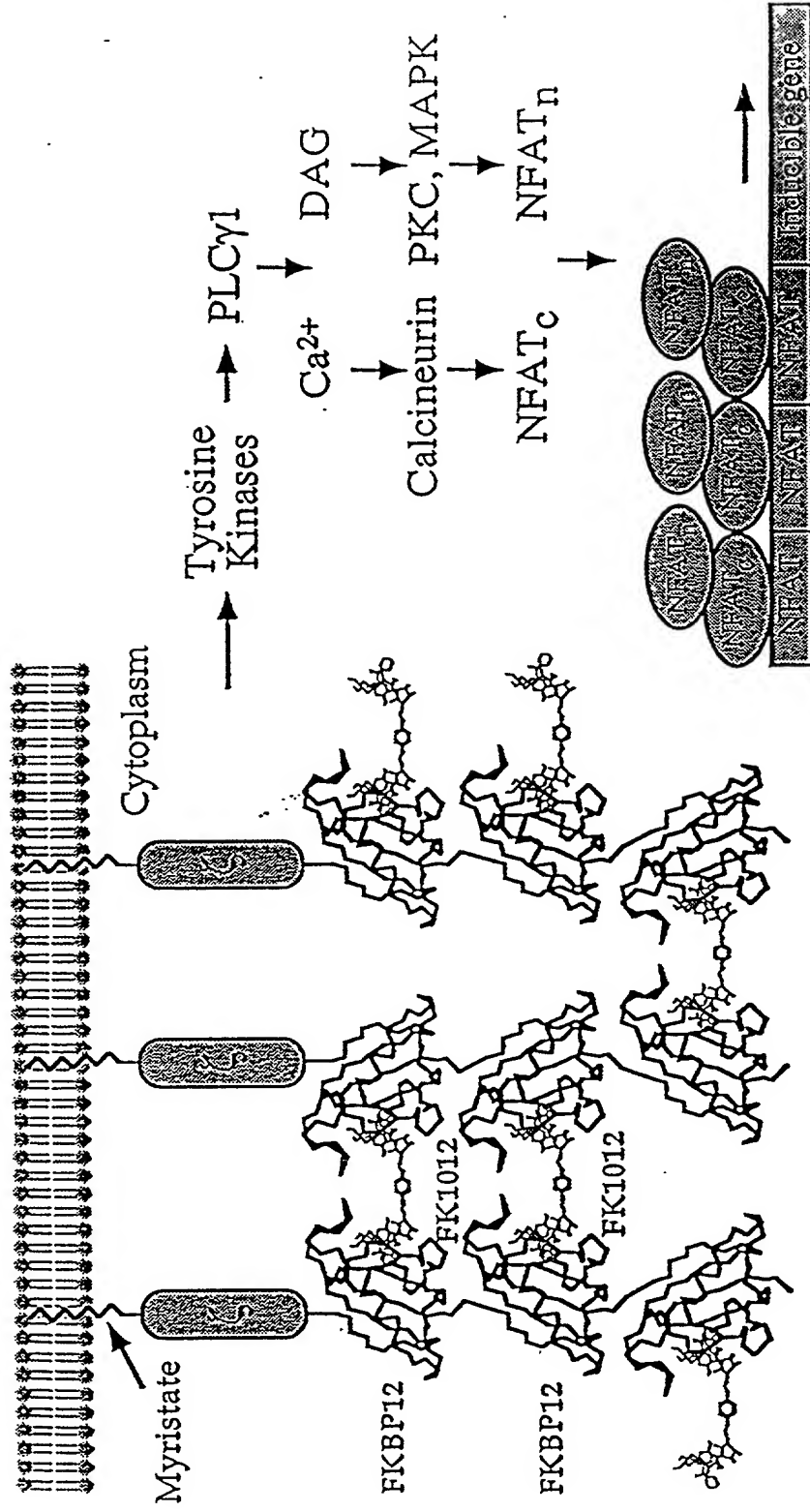


Figure 15/21

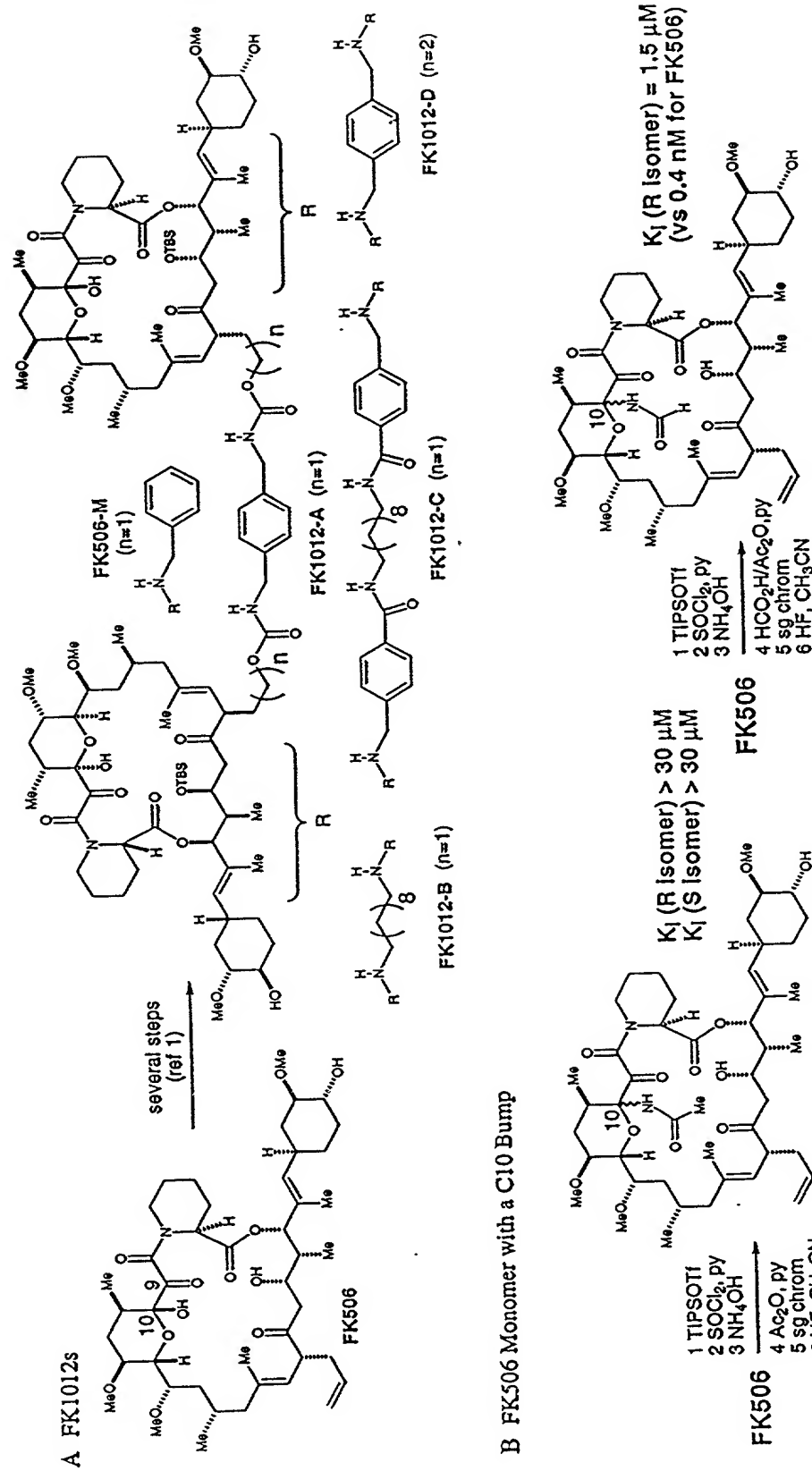
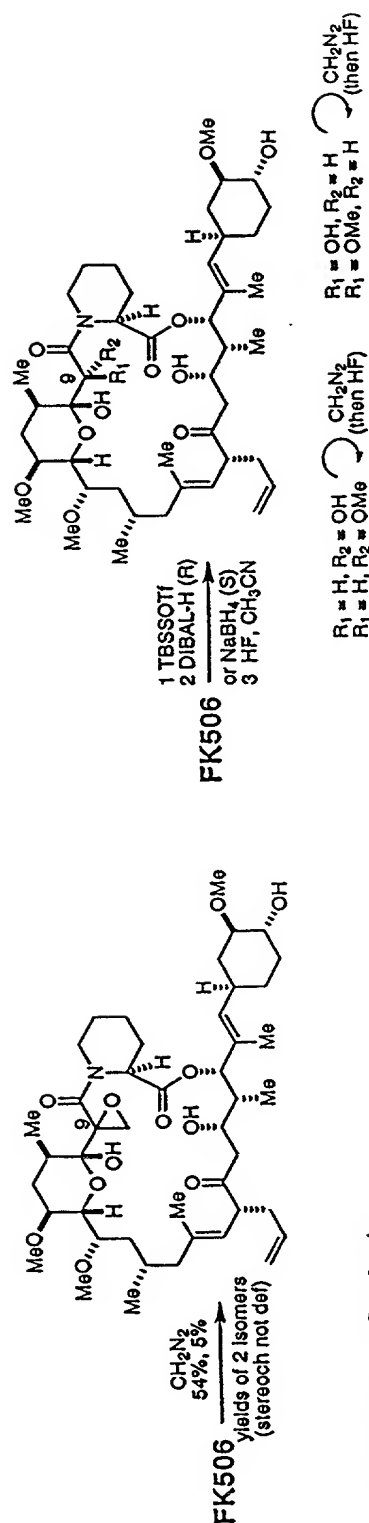


Figure 16 (#1)/21

C FK506 Monomer with a C9 Bump



D HED Reagent Synthesis

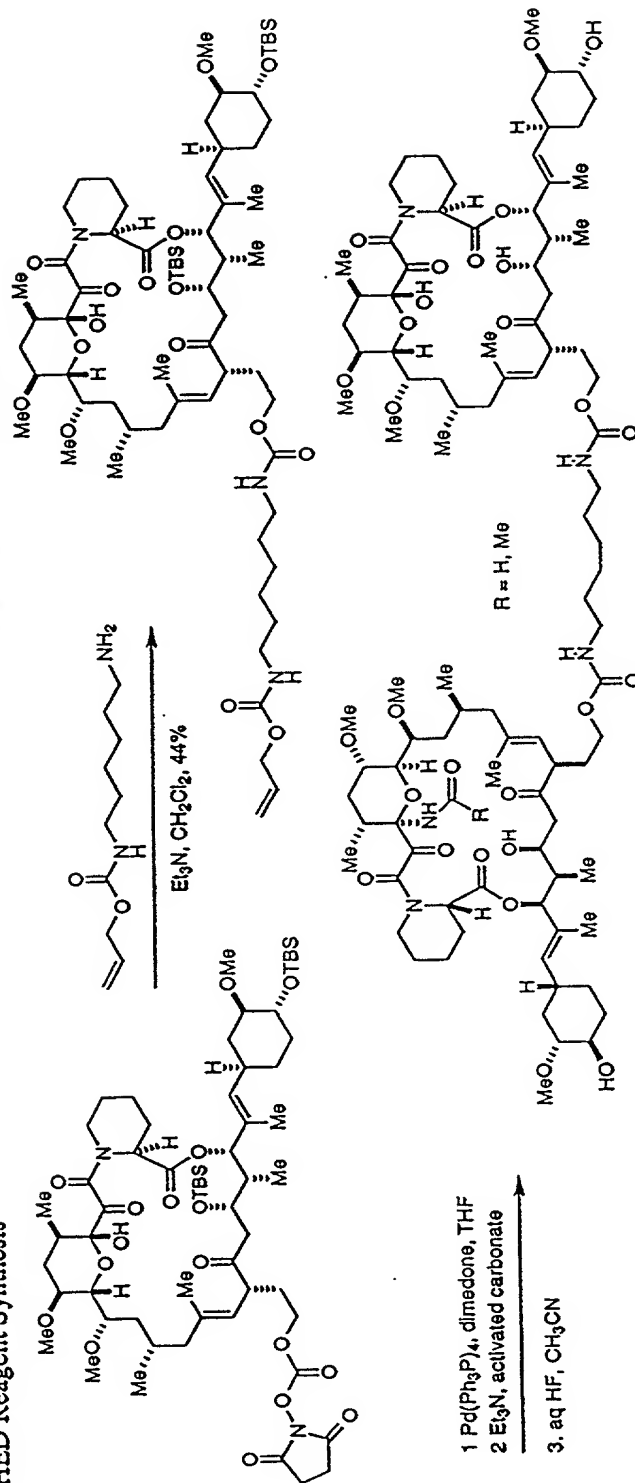


Figure 16 (#2)/21

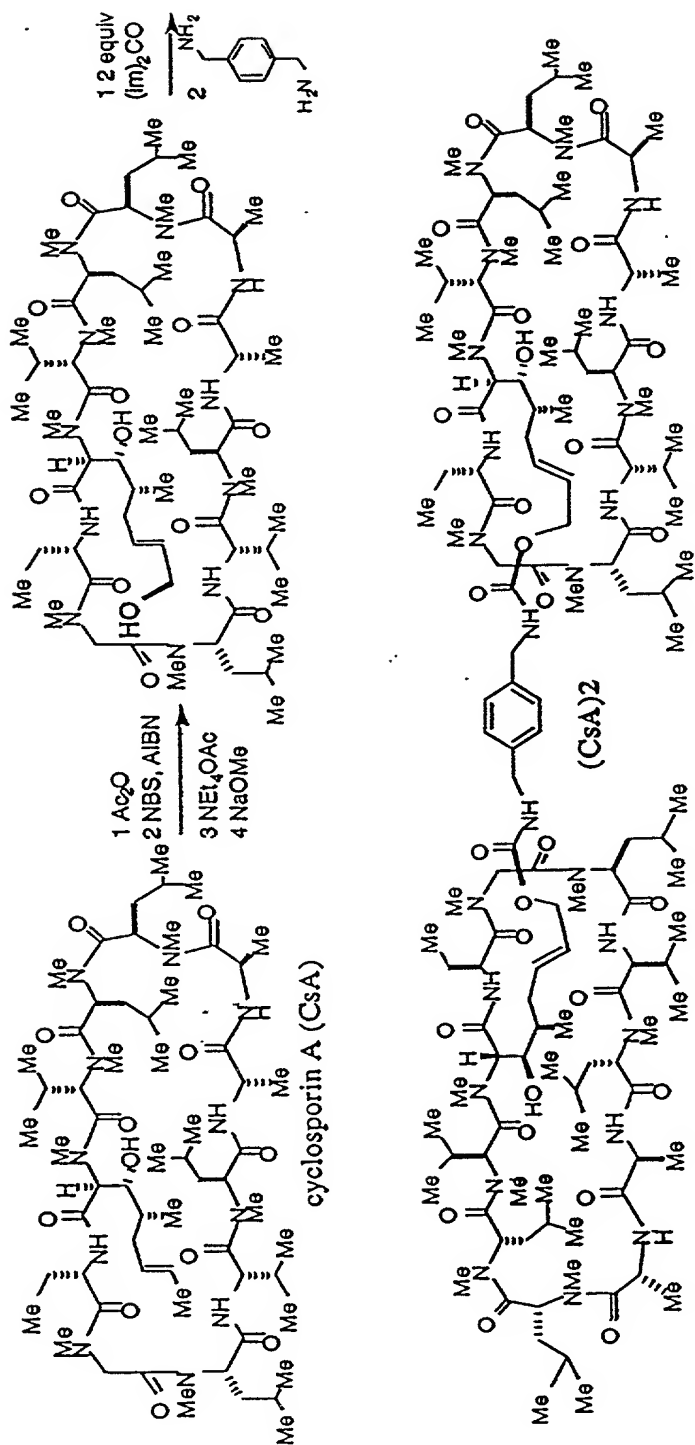


Figure 17/21

A cDNA construct

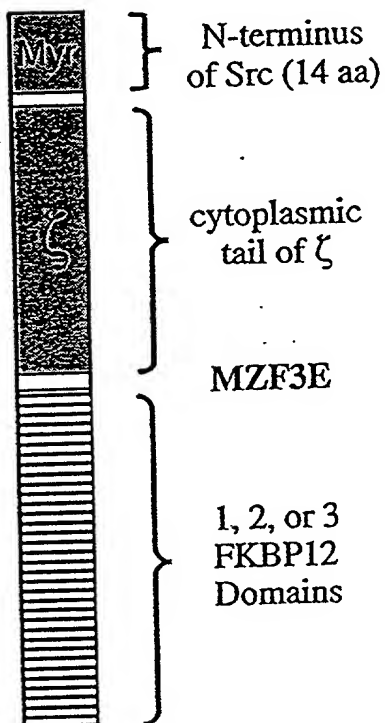


Figure 18A/21

B expressed protein

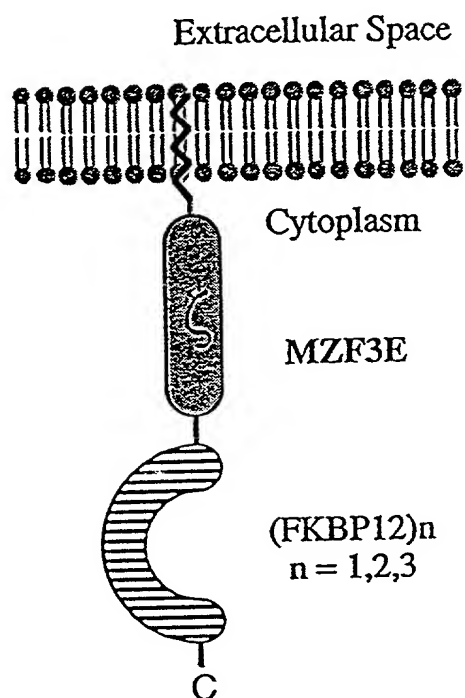


Figure 18B/21

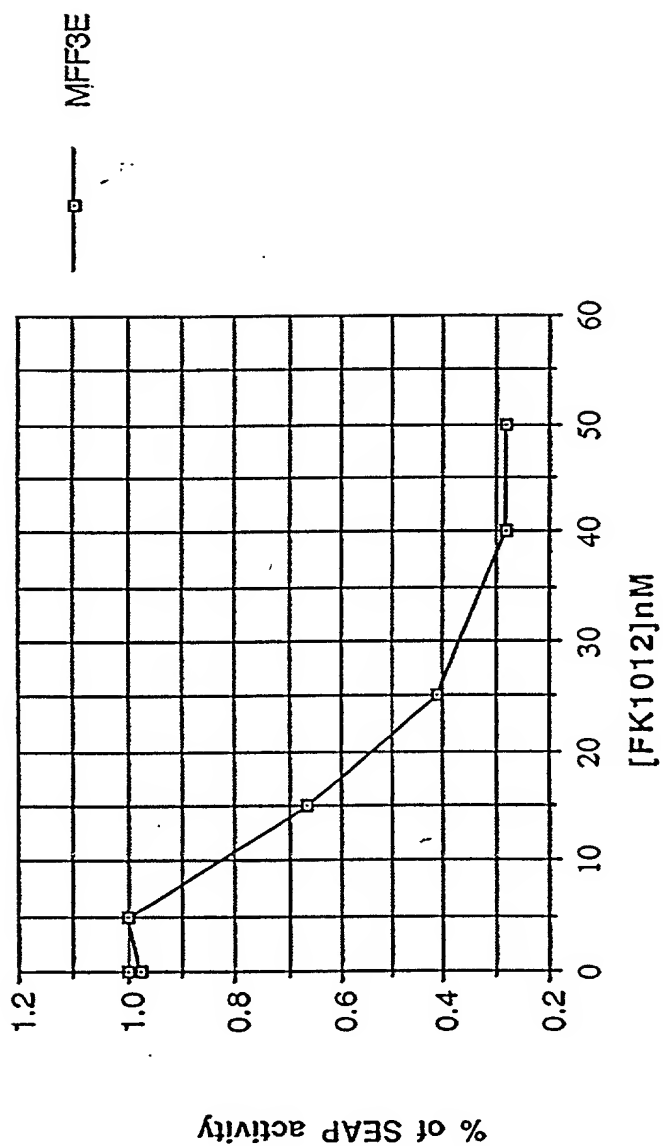


Figure 19/21

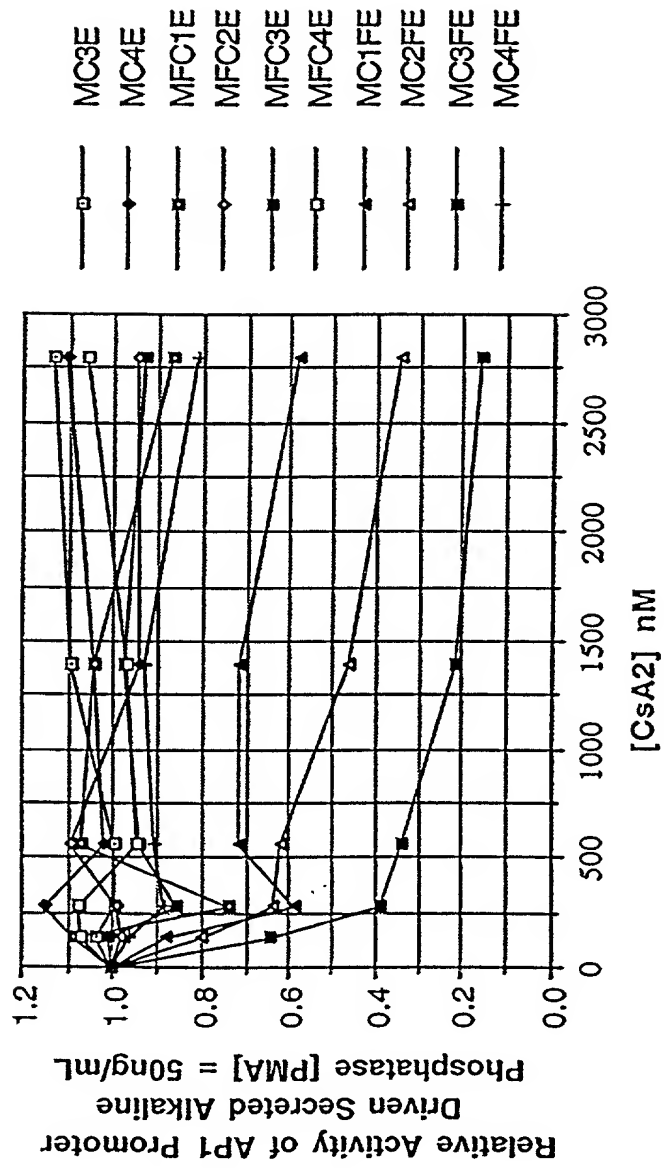


Figure 20A/21

	LD50 Jurkat Cells					Relative Protein Expression
	Myr	Fas	FKBP	FKBP	Ep	
A						
MFF3E	Myr	Fas	FKBP	FKBP	Ep	+
						15 nM
B						
MFC1E	Myr	Fas	CypC	CypC	Ep	-
MFC2E	Myr	Fas	CypC	CypC	Ep	-
MFC3E	Myr	Fas	CypC	CypC	Ep	-
MFC4E	Myr	Fas	CypC	CypC	Ep	-
MC1FE	Myr	CypC	Fas	Fas	Ep	+
MC2FE	Myr	CypC	CypC	Fas	Ep	+
MC3FE	Myr	CypC	CypC	CypC	Fas	+
MC4FE	Myr	CypC	CypC	CypC	Fas	+/-
MC3E	Myr	CypC	CypC	CypC	Ep	+++
MC4E	Myr	CypC	CypC	CypC	Ep	++++

Figure 20B/21

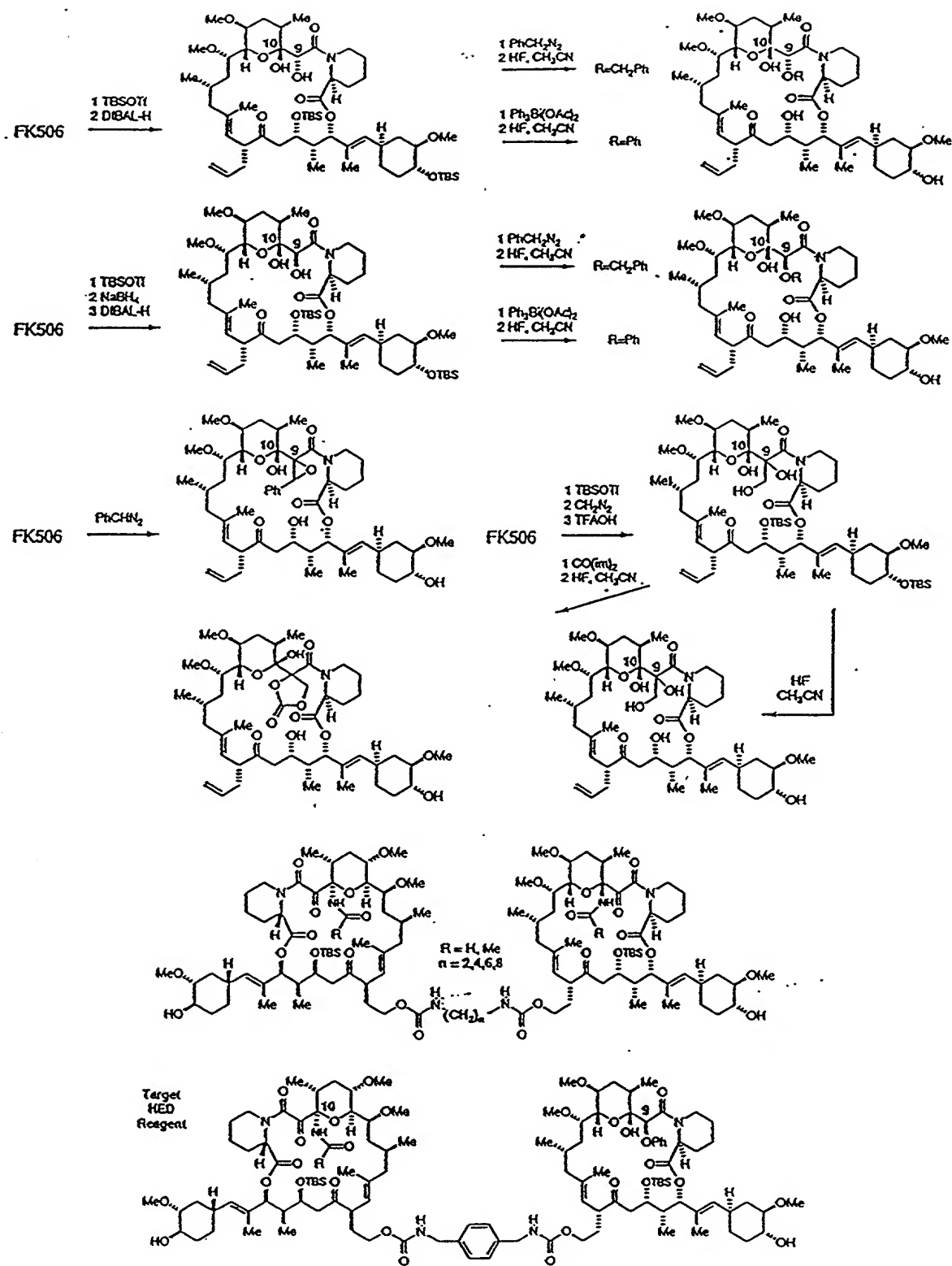


Figure 21/21